

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-158

PHARMACOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

IND: _____
Serial Number: 249
Type: Toxicology Information Amendment
Date of Submission: 6/26/02

Review Division: Special Pathogen and Immunologic Drug Products
HFD-590

Reviewer: Stephen G. Hundley, Ph.D., Pharmacologist

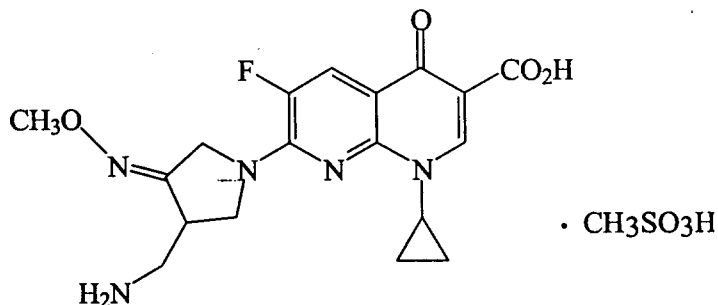
Review Completion Date: 1/10/03

Sponsor: GlaxoSmithKline
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101
Phone: 215 - 751- 3868

**APPEARS THIS WAY
ON ORIGINAL**

Drug Information

Name: Gemifloxacin (SB 265805 - S & LB20304a)
Drug Name: Factive® (Gemifloxacin mesylate) Tablets
Chemical Name: (±)-7-(3-aminomethyl-4-(Z)-methoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate
Molecular Formula: $C_{18}H_{20}FN_5O_4 \cdot CH_4O_3S$
Molecular Weight: 485.5 (mesylate salt)
Molecular Structure:



Indications: Treatment of community acquired pneumonia, acute exacerbation of chronic bronchitis, _____

BACKGROUND

Gemifloxacin is a fluoroquinolone that inhibits bacterial DNA gyrase activity and has broad antimicrobial activity. The proposed indications include community acquired pneumonia, acute exacerbation of chronic bronchitis, _____

_____ The proposed dosing regimen is 320 mg of gemifloxacin (active ingredient free of the mesylate salt) in tablet form, once daily, for periods ranging from three to ten days depending upon the indication.

The current submission contains studies that evaluated the inhibition of the cardiac IKr (HERG) potassium channel by gemifloxacin and the extent gemifloxacin prolonged the action potential duration in dog Purkinje fibers. These studies were reviewed and evaluated in this Pharmacology/Toxicology Review.

STUDY TITLES

Effect of SB-265805-S on the Human Cardiac IKr (HERG) Channel. Doc. ID: SB-265805/RSD-101MCC/1.

Effect of Selected Fluoroquinolones and Macrolide Antibiotics on the Human Cardiac IKr (HERG) Channel. Doc. ID: SB-265805/RSD-101MGD/1.

Effect of Moxifloxacin, Gatifloxacin, Levofloxacin, Grepafloxacin, and Sparfloxacin on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers. Doc. ID: General/RSD-1018F1/1.

SB-265805-S, Gatifloxacin, and Moxifloxacin Effect on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers. Doc. ID: SB-265805/RSD-101BT0/2.

STUDY REVIEWS

Effect of SB-265805-S on the Human Cardiac IKr (HERG) Channel. Doc. ID: SB-265805/RSD-101MCC/1.

Effect of Selected Fluoroquinolones and Macrolide Antibiotics on the Human Cardiac IKr (HERG) Channel. Doc. ID: SB-265805/RSD-101MGD/1.

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Both studies were conducted in accordance with GLP guidelines but were not subjected to Quality Assurance audits of the in-life laboratory work. The same methods and procedures were used in both studies. Human embryonic kidney cells (HEK 293) were transfected with the cDNA that encodes for the human IKr potassium channel (HERG). The transfected HEK 293 cell cultures express the IKr or rapidly activating delayed rectifier potassium channel. Inhibition of the cardiac IKr channel has been associated with prolongation of the QTc interval (repolarization of the cardiac action potential). The transfected ——— assay enables evaluation of the direct interaction of different drugs on this potassium channel.

Two pulsing frequencies, 0.1 and 3 Hz, were examined at hyperpolarization generated by -80 mV for 25 msec; followed by depolarization at $+40$ mV for 80 msec. The resulting action potential waves were generated by ——— techniques. The inhibition of the potassium channel by the different test drugs was evaluated at two incubation temperatures; 22°C and 34°C . The following compounds were evaluated: gemifloxacin (SB-265805-S), sparfloxacin, levofloxacin, grepafloxacin, gatifloxacin, moxifloxacin, azithromycin, clarithromycin, erythromycin, and terfenadine (positive control). Except for terfenadine, the concentrations of these drugs in cell culture ranged from ——— μM . Solubility problems limited the maximum gemifloxacin concentration to 500 μM . A minimum of three drug concentrations were utilized to mathematically generate IC_{50} values for each drug.

Data generated by the sponsor indicated that results did not differ for the two pulsing frequencies of 0.1 and 3 Hz for all compounds except gatifloxacin. Therefore, all of the IC_{50} values were generated at the 0.1 Hz pulsing frequency. The incubation temperature used to generate IC_{50} values was 34°C for gemifloxacin, moxifloxacin, azithromycin, clarithromycin, and erythromycin and 22°C for terfenadine, sparfloxacin, levofloxacin, grepafloxacin, and gatifloxacin. The IC_{50} values are listed in the following table.

Compound	IC_{50} (μM)	% Inhibition at 100 μM
Gemifloxacin	260	26
Sparfloxacin	37	66
Levofloxacin	827	5
Grepafloxacin	93	51
Gatifloxacin	329	16
Moxifloxacin	380	23
Azithromycin	1,500 (Extrapolated)	11
Clarithromycin	88	55
Erythromycin	388	22
Terfenadine	0.060	---

These data indicated that ——— and ——— (among the fluoroquinolones) shared similar IKr channel blocking characteristics based upon the IC_{50} estimations and the percent inhibition at 100 μM . The least active fluoroquinolone was ——— while the most active was ———. By comparison, the IC_{50} for the

_____ positive control was 0.60 μM , or approximately 600-fold more active than _____ the most active fluoroquinolone. These data do not suggest a strong signal for potential QTc prolongation activity for _____

Effect of _____ and _____ on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers. Doc. ID: General/RSD-1018F1/1.

SB-265805-S, _____ and _____ Effect on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers. Doc. ID: SB-265805/RSD-101BT0/2.

Several fluoroquinolones including _____ (SB-265805-S) were evaluated in the _____ assay for their effects upon action potential duration and maximum rate of depolarization. _____

_____ The procedures and methods were the same for the two studies. The study with SB-265805-S was conducted in accordance with GLP guidelines and audited by a Quality Assurance group.

[_____]
Stable
waveforms were typically achieved over a sixty-minute time interval. Base line waveforms were then established at stimulation frequencies of 0.5 and 1 Hz. Increasing concentrations of the fluoroquinolones (1, 10, and 100 μM) were then examined for their effects upon action potential duration at 90 and 60 percent repolarization (APD₉₀ & APD₆₀), upstroke amplitude (UA), resting membrane potential (RMP), and maximum rate of depolarization (MRD). Effects on these parameters were measured at both 0.5 and 1 Hz stimulation frequencies. The effect upon MRD was measured at the highest fluoroquinolone concentration (100 μM) at stimulation frequencies of 1 and 3 Hz. The postive control in both studies was _____ (50 μM).

None of the fluoroquinolones (including _____) exhibited effects upon UA, RMP, or MRD at any fluoroquinolone concentration and at any stimulation frequency. Each fluoroquinolone, except _____ increased APD₆₀ and APD₉₀ values (statistically significant percent increases) at the highest perfusion concentration of 100 μM at the 0.5 and 1 Hz stimulation frequencies. The increase observed with _____ was also statistically significant at the 10 μM concentration. The following chart lists the percent prolongation values for APD₉₀ at the 0.5 Hz stimulation frequency.

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Compound	% Prolongation of APD ₉₀ at 0.5 Hz Stimulation Frequency		
	1 μ M	10 μ M	100 μ M
[]	8 %	12 %	20 % *
	N.E.	N.E. / 9 %	30 % *
	N.E. / 4 %	N.E. / 13 %	25 % *
	N.E.	N.E.	52 % *
	N.E.	N.E.	3.3 %
	N.E.	24 % *	97 % *

N.E. indicates no effect on APD₉₀.

^a Compounds were evaluated in both studies, results from both studies listed if different.

* Statistically significant percent increase in prolongation.

Similar prolongation percentage results were obtained at the 1 Hz stimulation frequency for APD₉₀ and at both the 0.5 and 1 Hz stimulation frequencies for APD₆₀. _____ prolonged the APD₉₀ values by approximately 66 percent at the 0.5 Hz stimulation frequency. The percent prolongation at 1 Hz was less (49 percent) and consistent with the inverse relationship between prolongation induced by _____ and the stimulation frequency. None of the fluoroquinolones altered or reduced the MRD as the stimulation frequency was increased from 1 to 3 Hz indicating no interaction between these fluoroquinolones and the cardiac sodium channels.

COMMENTS AND EVALUATIONS

The _____ assay identifies compounds that directly interact with the cardiac IKr potassium channel. The sponsor developed sufficient data to calculate IC₅₀ (inhibition concentration) values for gemifloxacin and several comparator fluoroquinolones. The IC₅₀ for gemifloxacin (260 μ M) was similar to IC₅₀ values generated for gatifloxacin and moxifloxacin. Sparfloxacin was the most active fluoroquinolone in this assay and levofloxacin the least active. The IC₅₀ values for the comparator fluoroquinolones were consistent with IC₅₀ values generated for these fluoroquinolones by other sponsors. The sponsor also included a positive reference compound, terfenadine, to validate the assay.

Results from the dog _____ were consistent with observations from the _____ assay. The prolongation of APD₉₀ and APD₆₀ by gemifloxacin was similar to the prolongation generated at the same perfusion concentrations of moxifloxacin and gatifloxacin. As with the _____ potassium channel data, sparfloxacin exhibited the greatest activity in the _____ assay (prolongation of APD₉₀ & APD₆₀) whereas no statistically significant prolongation was generated by levofloxacin.

Data from the _____ and _____ assays indicated modest interactions between gemifloxacin and the IKr cardiac potassium channel. However, the responses observed at the highest cell culture or perfusion concentration used for gemifloxacin may

be of minor *in vivo* biological significance. The results from this study have no impact upon the ongoing clinical trials and proposed indications for (Factive®).

KEYWORDS:

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ON ORIGINAL**

Stephen G. Hundley, Ph.D., DABT
Pharmacology / Toxicology Reviewer HFD-590

Concurrences:
HFD-590/R.Albrecht/DDir
HFD-590/K.Hastings/TL

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**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Steve Hundley
1/16/03 07:49:05 AM
PHARMACOLOGIST

Kenneth Hastings
2/4/03 02:55:14 PM
PHARMACOLOGIST

Renata Albrecht
2/11/03 08:07:40 AM
MEDICAL OFFICER

**APPEARS THIS WAY
ON ORIGINAL**

NDA 21-158

FACTIVE[®] (gemifloxacin mesylate) 320mg Tablets

Action Date: December 15, 2000

**APPEARS THIS WAY
ON ORIGINAL**

TL: Leissa

MO: Powers, Alivisatos, Cox

CHM: M. Sloan

PCL: Ellis

MIC: Dionne

BPH: Colangelo

STT: Higgins, Dixon, Silliman

RPM: Kimzey

**APPEARS THIS WAY
ON ORIGINAL**

4. Kimzey

PHARMACOLOGY / TOXICOLOGY REVIEW AND EVALUATION OCT 18 2000

NDA: 21,158
Serial Number: 000
Type: Original NDA
Date of Submission: 12/16/99

APPEARS THIS WAY
ON ORIGINAL

Review Division: Special Pathogen and Immunologic Drug Products
HFD-590

Reviewer: Stephen G. Hundley, Ph.D., Pharmacologist

Review Completion Date: 10/16/00

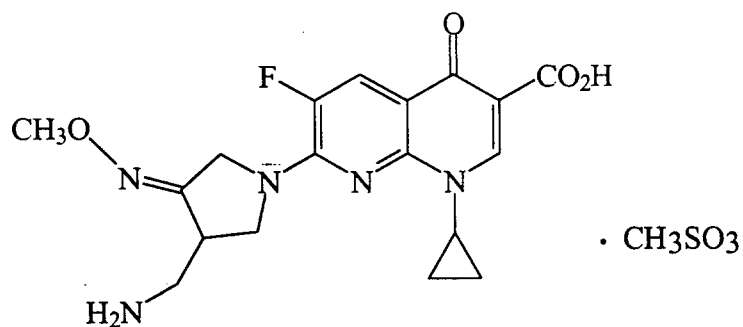
Sponsor: SmithKline Beecham Pharmaceuticals
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101

Phone: 215 - 751- 4000

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ON ORIGINAL

Drug Information

Name: Gemifloxacin (SB 265805 - S & LB20304a)
Drug Name: Factive®
Chemical Name: (±)-7-(3-aminomethyl-4-(Z)-methoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate
Molecular Formula: $C_{18}H_{20}FN_5O_4 \cdot CH_4O_3S$
Molecular Weight: 485.5 (mesylate salt)
Molecular Structure:



Drug Category: Antimicrobial - Fluoroquinolone
Related Submissions: IND's (oral) & (iv); DMF

Indications: Treatment of community acquired pneumonia, acute exacerbation of chronic bronchitis, _____

BACKGROUND

Gemifloxacin is a fluoroquinolone that inhibits bacterial DNA gyrase activity and has broad antimicrobial activity. The proposed indications include community acquired pneumonia, acute exacerbation of chronic bronchitis, _____

_____ The proposed dosing regimen is 320 mg of gemifloxacin (active ingredient free of the mesylate salt) in tablet form, once daily, for periods ranging from three to ten days depending upon the indication.

The Division of Anti-Infective Drug Products (HFD-520) is the primary review division for the Pharmacology/Toxicology portion of the NDA submission and requested that a joint Pharmacology/Toxicology review be conducted. The current review examines only the nonclinical metabolism and pharmacokinetic reports contained in the NDA submission. The primary Pharmacology/Toxicology Reviewer (Amy Ellis, Ph.D., Pharmacologist, HFD-520) examined all of the nonclinical pharmacology and toxicology studies submitted in support of this NDA and prepared the primary review.

NONCLINICAL METABOLISM AND PHARMACOKINETIC STUDIES

(^{GLP} Designates Audited Study)

SB Study Report Number SB-265805/RSD-100ZBR/1: An Investigatory Study to Examine Extraction Methodology for SB-265805 and Drug-Related Material from Biological Matrices.

SB Study Report Number SB-265805/RSD-101446/1: Summary Report, Preparation of Isotopically Labeled SB-265805.

SB Study Report Number SB-265805/RSD-100RVF/1: Determination of SB-265805 in Mouse Plasma by _____

SB Study Report Number SB-265805/RSD-100WFFV/1: Validation of an _____ Method with _____ for the Quantification of LB20304 in Rat Serum.^{GLP}

SB Study Report Number SB-265805/RSD-100MPZ/1: Determination of SB-265805 in Rat Plasma by _____

SB Study Report Number SB-265805/RSD-100RK5/2: Determination of SB-265805 (R,S) Enantiomers in Rat Plasma by _____

SB Study Report Number SB-265805/RSD-1010XC/1: Determination of SB-414000 (N-Acetyl SB-265805) in Rat Plasma by _____

SB Study Report Number SB-265805/RSD-100ZPX/1: Determination of SB-265805 in Rat Serum by _____

SB Study Report Number SB-265805/RSD-100MPX/2: Determination of SB-265805 in Rabbit Plasma by _____

SB Study Report Number SB-265805/RSD-100MZG/1: Determination of SB-265805 in Dog Plasma by _____

SB Study Report Number SB-265805/RSD-100RK6/1: Determination of SB-265805 (R,S) Enantiomers in Dog Plasma by _____

SB Study Report Number SB-265805/RSD-10108L/1: In Vitro Stability and In Vivo Interconversion Study.

SB Study Report Number SB-265805/RSD-1011R3/1: Plasma Concentrations and Excretion of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805-S to Male and Female Hairless Mice at a Target Dose of 100 mg free base/kg. GLP

SB Study Report Number SB-265805/RSD-100XLK/1: A Study to Determine the Pharmacokinetics of SB-265805 Following Intraperitoneal Administration of SB-265805-S at a Nominal Dose Level of 50 mg/kg (as the Mesylate Salt) in the Mouse. GLP

SB Study Report Number SB-265805/RSD-1011XK/1: The Metabolism of [14C]SB-265805 in the Hairless Mouse. GLP

SB Study Report Number SB-265805/RSD-100TVN/2: A Preliminary Study to Characterise Drug-Related Material in Urine, Bile, Faeces, and Plasma Following a Single Oral Administration of [14C]SB-265805 to the Male Rat at a Target Dose Level of 20 mg free base/kg, and to Assess Excretion of Compound Related Material Following a Single Intravenous Administration at a Target Dose of 10 mg free base/kg.

SB Study Report Number SB-265805/RSD-100XCS/1: Elimination of Drug-Related Material Following Single Doses of [14C]SB-265805-S to Male and Female Rats. GLP

SB Study Report Number SB-265805/RSD-100TS0/1: An Investigative Study to Determine the Pharmacokinetics of the R- and S-Enantiomers of SB-265805 in the Rat Following both Intravenous and Oral Administration of Racemic RS-SB-265805 at Nominal Dose Levels of 10 and 30 mg free base/kg, Respectively. GLP

SB Study Report Number SB-265805/RSD-1013DJ/1: SB-265805 (Gemifloxacin): A Study to Determine the Pharmacokinetics of Gemifloxacin and SB-414000 (N-Acetyl

Gemifloxacin) Following Oral Administration of Gemifloxacin Mesylate at Nominal Doses of 210 and 750 mg/kg (as the mesylate salt) in the Rat. ^{GLP}

SB Study Report Number SB-265805/RSD-100V86/3: A 14-Day Oral Study in Rats to Investigate the Pharmacokinetics of SB-265805 and the Effect on Hepatic Levels of Cytochrome P450 and Related Parameters. ^{GLP}

SB Study Report Number SB-265805/RSD-100XCM/1: [14C]SB-265805-S: Quantitative Whole-Body Autoradiography Following Single Oral Administration (210 mg/kg) to the Male Pigmented Rat. ^{GLP}

SB Study Report Number SB-265805/RSD-100XCP/2: Placental Transfer of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805-S to the Pregnant Rat at a Nominal Dose Level of 270 mg/kg (217 mg free base/kg). ^{GLP}

SB Study Report Number SB-265805/RSD-100XCR/1: Milk Secretion of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805 to the Lactating Rat at a Nominal Dose Level of 30 mg/kg (24 mg free base/kg). ^{GLP}

SB Study Report Number SB-265805/RSD-1011R4/1: Biliary and Plasma Concentrations of Drug-Related Material Following Single and 5-Day Repeat Intravenous Administration of [14C]SB-265805-S to Male Bile Duct-Cannulated Beagle Dogs at a Target Dose of 30 mg free base/kg/day. ^{GLP}

SB Study Report Number SB-265805/RSD-1011XJ/1: An Investigation of the Plasma Concentrations of SB-265805 and the Biliary Metabolite Profiles Following Single and 5-Day Repeat Intravenous Administration of [14C]SB-265805-S to Male Bile-Duct Cannulated Dogs at a Target Dose of 30 mg free base/kg. ^{GLP}

SB Study Report Number SB-265805/RSD-100SKR/2: A Preliminary Study to Investigate the Absorption, Excretion, and Biliary Secretion of Drug-Related Material in the Dog Following a Single Oral Administration of [14C]SB-265805 at a Target Dose of 24 mg free base/kg.

SB Study Report Number SB-265805/RSD-100XCK/1: Elimination of Drug-Related Material Following Single Doses of [14C]SB-265805-S to Male and Female Dogs. ^{GLP}

SB Study Report Number SB-265805/RSD-100XFZ/1: Biotransformation of [14C]SB-265805 in Rat and Dog. ^{GLP}

SB Study Report Number SB-265805/RSD-100T6Z/1: An Investigative Study to Determine the Pharmacokinetics of the R- and S-Enantiomers of SB-265805 Following both Intravenous and Oral Administration of SB-265805-S at a Nominal Dose Level of 10 mg/kg (as the free base) to the Male Beagle Dog. ^{GLP}

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ON ORIGINAL**

SB Study Report Number SB-265805/RSD-100WK7/1: A Study in the Beagle Dog to Determine the Intravenous Pharmacokinetics of SB-265805 Following a 30 Minute Intravenous Infusion of SB-265805 at Nominal Dose Levels of 10 and 30 mg/kg (as the free base). ^{GLP}

SB Study Report Number SB-265805/RSD-1013MS/1: A Study in the Beagle Dog to Determine the Concentrations in Cardiac Tissue Following a 30 Minute Intravenous Infusion of SB-265805 at a Nominal Dose Level of 30 mg/kg (as the free base). ^{GLP}

SB Study Report Number SB-265805/RSD-100XG0/2: The Effect of SB-265805-S on Hepatic Levels of Cytochrome P450 and Related Parameters in Beagle Dogs after Oral Administration at 0, 5, 30, and 120 mg/kg/day for 13 Weeks. ^{GLP}

SB Study Report Number SB-265805/RSD-XFX/1: A Preliminary *in vitro* Investigation into the Biotransformation of SB-265805.

SB Study Report Number SB-265805/RSD-100W5S/1: Characterisation of the Metabolism of [14C]SB-265805 in Whole Cell Liver Tissue Models.

SB Study Report Number SB-265805/RSD-100XCV/1: The Metabolism of [14C]SB-265805 in Whole Cell Liver Systems from Rabbit and Mouse. ^{GLP}

SB Study Report Number SB-265805/RSD-1013M3/1: An *in vitro* Investigation into the N-Acetylation of SB-265805. ^{GLP}

SB Study Report Number SB-265805/RSD-10158N/1: A Preliminary Investigation into the Cytochrome P450 Inhibitory Potential of SB-265805.

SB Study Report Number SB-265805/RSD-100ZHK/2: Evaluation of Racemic SB-265805 and its Individual Enantiomers as Inhibitors of Human P450 Enzymes *in vitro*. ^{GLP}

SB Study Report Number SB-265805/RSD-100XCN/2: The *in vitro* Blood/Plasma Partitioning of [14C]SB-265805 in Rat, Dog, and Man. ^{GLP}

SB Study Report Number SB-265805/RSD-100T54/1: The *in vitro* Protein Binding of the R and S Enantiomers of SB-265805 in Rat, Mouse, Dog, and Human Plasma. ^{GLP}

SB Study Report Number SB-265805/RSD-1010V9/1: Investigation of SB-265805 (Fluoroquinolone) Permeability *in vitro* Across Various Intestinal Models.

Previously Reviewed Metabolism and Pharmacokinetic Studies (IND Submissions)

SB Study Report Number SB-265805/RSD-100M9Z/2: Validation of a Bioanalytical Method for the Measurement of LB20304a in Rat Plasma.

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SB Study Report Number SB-265805/RSD-100M9W/1: Validation of the Analytical Procedure for the Determination of LB-20304a in Rat Serum using _____ with

SB Study Report Number SB-265805/RSD-100MB0/3: Validation of a Bioanalytical Method for the Measurement of LB20304a in Dog Plasma.

SB Study Report Number SB-265805/RSD-100M9X/1: Validation of the Analytical Procedure for the Determination of LB-20304a in Dog Serum using _____ with

SB Study Report Number SB-265805/RSD-100MB3/2: 14C-LB20304a: Pharmacokinetics, Tissue Distribution, Metabolism, and Excretion Studies in the Rat.

SB Study Report Number SB-265805/RSD-100MB1/1: LB20304: Pharmacokinetics in Rats and Dogs.

SB Study Report Number SB-265805/RSD-100MB4/1: LB20304: Metabolism.

NONCLINICAL METABOLISM AND PHARMACOKINETICS STUDIES REVIEWS

SB Study Report Number SB-265805/RSD-100ZBR/1: An Investigatory Study to Examine Extraction Methodology for SB-265805 and Drug-Related Material from Biological Matrices.

SB Study Report Number SB-265805/RSD-101446/1: Summary Report, Preparation of Isotopically Labeled SB-265805.

SB Study Report Number SB-265805/RSD-100RVF/1: Determination of SB-265805 in Mouse Plasma by _____

SB Study Report Number SB-265805/RSD-100WFV/1: Validation of an _____ Method with _____ for the Quantification of LB20304 in Rat Serum.^{GLP}

SB Study Report Number SB-265805/RSD-100MPZ/1: Determination of SB-265805 in Rat Plasma by _____

SB Study Report Number SB-265805/RSD-100RK5/2: Determination of SB-265805 (R,S) Enantiomers in Rat Plasma by _____

SB Study Report Number SB-265805/RSD-1010XC/1: Determination of SB-414000 (N-acetyl SB-265805) in Rat Plasma by _____

SB Study Report Number SB-265805/RSD-100ZPX/1: Determination of SB-265805 in Rat Serum by _____

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SB Study Report Number SB-265805/RSD-100MPX/2: Determination of SB-265805 in Rabbit Plasma by _____

SB Study Report Number SB-265805/RSD-100MZG/1: Determination of SB-265805 in Dog Plasma by _____

SB Study Report Number SB-265805/RSD-100RK6/1: Determination of SB-265805 (R,S) Enantiomers in Dog Plasma by _____

SB Study Report Number SB-265805/RSD-10108L/1: In Vitro Stability and In Vivo Interconversion Study.

The studies cited above can be broadly classified as bioanalytical procedures development research. The summary report on the preparation of radiolabelled SB-265805 was not subject to critical review as it summarized final analytical purity results for [^{14}C]-SB-265805 synthesized by the sponsor. The report on extraction methodology for radioactive material derived from [^{14}C]-SB-265805 contained in rat plasma and fecal samples also contained summarized results and was not critically reviewed. The liquid extraction procedure of fecal homogenates was at least 90+% effective and the solid phase extraction of plasma was 90 to 93% effective in removing radioactive compounds for analysis. The solid phase extraction procedure was also 90+% effective in removing radioactive compounds from dog plasma. The extraction procedures cited in the report were typical of methods commonly used to extract radiolabelled parent and metabolites from biological matrices such as plasma, feces, and urine for _____ analysis of metabolic profiles.

The remainder of the studies (with one exception) detailed various analytical procedures for analysis of SB-265805 concentrations in plasma or serum from experimental animals (mice, rats, rabbits, and dogs). All of the plasma analytical procedures in these studies involved precipitation of plasma protein by the addition of acetonitrile with subsequent centrifugation followed by removal of the acetonitrile supernatant for _____ analysis. This procedure effectively extracted all of the SB-265805 present in the plasma and did not distinguish between free and protein bound SB-265805. The _____ analytical method was validated in rats (GLP study audited by a Quality Assurance group). The procedure utilized a reverse phase _____ system that included a standard reverse-phase column (PLRP- C_{18} , 5 μm , 50 x 4.6 mm i.d.) and an isocratic solvent system (76:24, 0.1% trifluoroacetic acid : acetonitrile). SB-265805 was detected by _____ (337 nm excitation wavelength and 405 nm emission wavelength). The lower limit of quantitation (LLQ) was _____ μg SB-265805 per ml serum and the linear range was from _____ μg SB-265805 per ml serum (based upon an internal standard quantitation method). Validation of the analytical procedure included selectivity, linearity of response, coefficients of variation for precision and accuracy, reproducibility, and analyte carry over.

The sponsor subsequently developed a more sensitive analytical procedure based upon detection by _____ A reverse phase _____ system was utilized to

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effect analyte separation in the plasma extract. The — column was the same as described in the previous paragraph while the isocratic solvent system consisted of 70:30, 0.01 M ammonium acetate at pH 2.5 : acetonitrile. The — was interfaced with an — system in the positive ion mode that analyzed the positive ion mass spectrum for SB-265805 ($m/z = 390.1$) and the daughter ion ($m/z = 312.9$). The LLQ for SB-265805 with this procedure was — SB-265805 per ml plasma and the linear range for detection was — SB-265805 per ml plasma (based upon an internal standard quantitation method). This procedure was validated in dog and rat plasma as described in the previous paragraph for the — procedure. The — procedure for SB-265805 was also validated for mouse and rabbit plasma and rat serum.

Additionally, an — procedure was used to assay for concentrations of the N-acetyl metabolite of SB-265805 in rat plasma. The — system included a reverse phase C₈ column and a 65:35, 0.01 M ammonium acetate at pH 2.5 : acetonitrile solvent system. The LLQ was — ng N-acetyl-SB-265805 per ml plasma and the linear range for detection was — ng N-acetyl-SB-265805 per ml plasma (based upon an internal standard quantitation method). This procedure was validated in accordance with procedures cited for the previous analytical methods.

SB-265805 exists as a racemic mixture of the R- and S-enantiomers and the sponsor developed — analytical techniques to separate and quantitate the two enantiomers. The submission includes two reports validating these procedures in rat and dog plasma. The — system included a chiral analytical column (Crownpak CR(+), 150 mm X 4 mm i.d.) and an isocratic solvent system consisting of 0.01 M ammonium acetate at pH 2.0 : methanol (60:40). The — procedure was similar to those previously described. This analytical system achieved baseline separation of the two enantiomers with retention times of 3.6 and 5.0 minutes for the R- and S-enantiomers, respectively. The LLQ was — ng/ml and the linear range of detection was — ng/ml for each enantiomer. This system was validated in accordance with procedures used to validate the other analytical systems cited in this review. The sponsor utilized a similar — system with a chiral column but with — detection to determine if interconversion occurred between the R- and S-enantiomers during *in vitro* incubations and following *in vivo* administration. No conversion occurred after 24 hours of incubation in human plasma or under acidic aqueous (pH 2.0) conditions. No conversion occurred in plasma or urine samples from rats dosed with SB-265805.

SB Study Report Number SB-265805/RSD-1011R3/1: Plasma Concentrations and Excretion of Drug-Related Material Following a Single Oral Administration of [¹⁴C]SB-265805-S to Male and Female Hairless Mice at a Target Dose of 100 mg free base/kg.
GLP

SB Study Report Number SB-265805/RSD-100XLK/1: A Study to Determine the Pharmacokinetics of SB-265805 Following Intraperitoneal Administration of SB-265805-S at a Nominal Dose Level of 50 mg/kg (as the Mesylate Salt) in the Mouse.
GLP

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SB Study Report Number SB-265805/RSD-1011XK/1: The Metabolism of [¹⁴C]SB-265805 in the Hairless Mouse. ^{GLP}

Male and female hairless mice (SKH-hrBR) received [¹⁴C]SB-265805 by oral administration at a 100 mg/kg dose level. This dose level was selected because it was a no-effect dose level in a phototoxicity study conducted with hairless mice. Urine and feces were collected at 24 hour intervals for 96 hours following dosing. At the end of 96 hours approximately 93 percent of the administered radioactivity from SB-265805 was eliminated in the feces while approximately 3 percent was excreted in urine. The carcass and G.I. tract contained less than 0.05 percent of the administered radioactive substance. The initial 24-hour collection contained 99 percent of the total fecal excretion and 96 percent of the urinary excretion of radioactive compounds derived from [¹⁴C]SB-265805. Blood samples were collected from a separate group of male mice at 0.5, 2, and 4 hours after dosing. The data were expressed as µg equivalents of [¹⁴C]SB-265805 per ml of plasma (based upon total radioactivity in plasma). The plasma values (from pooled samples) were 3.98, 1.7, and 0.27 µg equivalents per ml at the 0.5-, 2-, and 4-hour timepoints, respectively. The excretion and plasma data indicated rapid elimination of orally dosed SB-265805 from hairless mice (both sexes).

Metabolite profiles were subsequently developed by _____ detection techniques for the urine, fecal, and plasma samples from the study with hairless mice. The fecal extraction procedure was essentially quantitative while the urine samples were diluted and then analyzed (no extraction was involved). The plasma extraction procedure removed approximately 80 percent of the total plasma radioactivity. Similar plasma extraction procedures quantitatively removed SB-265805, therefore the residual or non-extracted plasma radioactivity represented a metabolite or metabolites of SB-265805. _____ and _____ analyses determined that 89 percent of the fecal and 81 to 83 percent of the urinary radioactivity was unchanged SB-265805. The only identified metabolite common to urine and feces was the E-isomer of SB-265805 (2 to 3 percent of fecal radioactivity and 3 to 5 percent of urinary radioactivity). Urine also contained the acyl glucuronide of SB-265805 (representing 4 to 9 percent of urinary radioactivity) while the fecal extracts contained N-acetyl SB-265805 (representing 2 to 3 percent of the fecal radioactivity). These data indicated that approximately 85 percent of the total dose was unchanged SB-265805. The E-isomer of SB-265805 and N-acetyl SB-265805 each represented 3 percent of the total dose. The acyl glucuronide of SB-265805 only represented 0.2 percent of the total dose.

Analysis of the plasma radioactivity indicated _____ percent of the extracted radioactivity was unchanged SB-265805 at the 0.5- and 2-hour timepoints while the acyl glucuronide represented _____ percent. The E-isomer of SB-265805 was also present and accounted for 6 to 9 percent of the extracted radioactivity. N-acetyl SB-265805 was not present in the 0.5 hour plasma sample and represented only 0.6 percent of the extracted radioactivity at the 2-hour timepoint. These data indicated that although the acyl glucuronide of SB-265805 was the most prominent SB-265805 metabolite in plasma it was not observed in the feces and was only _____ of the urinary excretion.

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Plasma pharmacokinetics were determined in male and female CD-1 mice following single intraperitoneal injections of SB-265805 at a 50 mg/kg dose level. This study was conducted in order to estimate systemic exposure at this dose level from a previously conducted mouse micronucleus test. The post-dosing blood sampling timepoints were 2, 10, and 30 minutes; then 1, 2, 5, 10, and 24 hours post-dosing. Plasma samples were analyzed by _____, procedures for SB-265805. No differences were observed between male and female mice. The C_{max} and AUC values were 4.2 µg/ml and 11.5 µg • hr/ml, respectively. Plasma SB-265805 concentrations were above the LLQ of _____ ng/ml at the 24-hour timepoint and had decreased approximately 380-fold from the C_{max} levels. The sponsor did not determine plasma half-life values for SB-265805.

SB Study Report Number SB-265805/RSD-100TVN/2: A Preliminary Study to Characterise Drug-Related Material in Urine, Bile, Faeces, and Plasma Following a Single Oral Administration of [¹⁴C]SB-265805 to the Male Rat at a Target Dose Level of 20 mg free base/kg, and to Assess Excretion of Compound Related Material Following a Single Intravenous Administration at a Target Dose of 10 mg free base/kg.

SB Study Report Number SB-265805/RSD-100XCS/1: Elimination of Drug-Related Material Following Single Doses of [¹⁴C]SB-265805-S to Male and Female Rats. ^{GLP}

A preliminary metabolism/excretion study was conducted with bile-duct cannulated and non-cannulated male CD rats at dose levels of 10 or 20 mg [¹⁴C]SB-265805 per kg body weight. The 10 mg/kg dose was by *iv* administration and the 20 mg/kg dose was administered orally. Bile-duct cannulated rats excreted approximately 14 percent of the orally administered 20 mg/kg dose in the urine, 70 percent in the feces, and 5 percent in the bile. The excretion profile for the non-cannulated rats was 19 percent in urine and 73 percent in feces. Most of the excretion occurred in the initial 24 hours after dosing to the cannulated and non-cannulated rats.

Bile-duct cannulated rats administered a 10 mg/kg intravenous dose excreted 40 percent of the dose in urine, 30 percent in feces, and 12 percent in bile during the initial 24 hours after dosing. These data indicated a substantial potential for secretion of [¹⁴C]SB-265805-derived radioactive compounds from the intestinal epithelium into the intestinal lumen for eventual fecal elimination. Bile-duct cannulation removed any biliary contribution to fecal elimination. These preliminary oral and *iv* studies suggested limited oral bioavailability for SB-265805 in rats. An accurate measure of bioavailability was difficult due to the number of animals in each dosing routine (one or two) and the potential contribution to fecal excretion by secretion of SB-265805 and its metabolites by the intestinal epithelium into the gut lumen.

_____ analysis with _____ and _____ detection indicated that the primary excretion product was unchanged SB-265805 (approximately 90 percent of the urinary excretion and 95 percent of the extracted fecal radioactivity). The E-isomer of SB-265805 was present in urine samples and fecal extracts (6 and 3 percent, respectively). N-acetyl SB-265805 was also detected in urine samples and fecal extracts

at about 1 or 2 percent relative abundance. Urine also contained the acyl glucuronide of SB-265805 and desmethylamine SB-265805 (approximately 1 percent of the total urinary amounts for each compound). The most prominent metabolite in bile was the acyl glucuronide of SB-265805 (74 percent relative abundance). Equivalent amounts of SB-265805 and the N-acetyl, acyl glucuronide of SB-265805 were present in bile (9 to 11 percent relative abundance). Approximately 80 percent of the radioactivity extracted from the plasma was SB-265805 while 7 percent was the E-isomer of SB-265805. The acyl glucuronide of SB-265805 represented 4 to 6 percent and N-acetyl SB-265805 represented from 2 to 4 percent of the extracted radioactivity. Plasma also contained a metabolite tentatively identified as the N-hydroxy derivative of SB-265805 which increased in relative abundance with sample time (2 to 8 percent as the sample time increased from 1 to 6 hours post-dosing).

The relative abundance data for SB-265805 and its metabolites are tentative due to the small number of animals involved at each dosing routine. In addition, the fecal extraction procedure outlined in this study was only 52 to 80 percent efficient, therefore a substantial amount of excreted radioactivity was not analyzed by _____ procedures. Similarly, the extraction procedure for plasma removed 80 percent of the plasma radioactivity. The remaining 20 percent in plasma was not available for _____ analysis and may reflect reduced extraction efficiency for certain metabolites or non-extractable radioactivity (macromolecular bound radioactivity).

Excretion of [^{14}C]SB-265805-derived radioactivity was determined in male and female rats following oral administration of a single 168 mg/kg dose of radiolabelled SB-265805. Excretion was also monitored in bile-duct cannulated male rats dosed orally at the 168 mg/kg level or intravenously at 10 mg/kg. Urine and feces were collected at 24-hour intervals for 96 hours following dosing. Approximately 90 percent of the dosed radioactivity was excreted in feces from males and females receiving the 168 mg/kg oral dose (non-cannulated animals). Urinary excretion from these animals was approximately 9 percent of the administered radioactive dose. Only a fraction of a percent of the original dose remained in the carcass at the 96-hour terminal sacrifice. Similar data were obtained from bile-duct cannulated male rats receiving the 168 mg/kg oral dose. Fecal excretion represented 86 percent of the dose and bile contained approximately 4 percent of the administered radioactivity. However, in cannulated male rats dosed *iv* with 10 mg [^{14}C]SB-265805/kg body weight, 46 percent of the administered radioactivity was excreted in urine, 33 percent in feces, and approximately 12 percent in bile. These data confirmed the secretion of SB-25805-derived radiolabelled compounds from the intestinal epithelium into the intestinal lumen for fecal elimination.

Plasma radioactivity was monitored in separate groups of male and female rats dosed orally at the 168 mg/kg dose level. The C_{max} for ^{14}C radioactivity occurred at 2 hours post-dosing and was _____ μg equivalents of [^{14}C]SB-265805 per ml plasma for males and females, respectively. The radioactivity levels were below the limit of accurate quantitation (_____ μg equivalents/ml) by 24 hours post-dosing.

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SB Study Report Number SB-265805/RSD-100TS0/1: An Investigative Study to Determine the Pharmacokinetics of the R- and S-Enantiomers of SB-265805 in the Rat Following both Intravenous and Oral Administration of Racemic RS-SB-265805 at Nominal Dose Levels of 10 and 30 mg free base/kg, Respectively. ^{GLP}

SB Study Report Number SB-265805/RSD-1013DJ/1: SB-265805 (Gemifloxacin): A Study to Determine the Pharmacokinetics of Gemifloxacin and SB-414000 (N-Acetyl Gemifloxacin) Following Oral Administration of Gemifloxacin Mesylate at Nominal Doses of 210 and 750 mg/kg (as the mesylate salt) in the Rat. ^{GLP}

Pharmacokinetic patterns were developed for the R- and S-enantiomers of SB-265805 in jugular-vein cannulated male CD rats following a single 10 mg/kg *iv* infusion and a single 30 mg/kg oral administration of racemic SB-265805. The R- and S- enantiomers were assayed using a _____ and _____ techniques similar to those described in previously reviewed studies (100RK5/2 and 100RK6/1). The LLQ for this procedure was approximately _____ ng/ml. Sequential blood samples were drawn from each of four animals for 24 hours following the *iv* infusion and the oral dose. The following pharmacokinetic data were developed for the two enantiomers.

	10 mg/kg IV Infusion		30 mg/kg Oral Dose	
	R-SB-265805	S-SB-265805	R-SB-265805	S-SB-265805
C _{max} (ng/ml)	1200	970	160	140
AUC (ng • hr/ml)	2200	2000	715	640
t _{1/2} (hours)	1.5	1.5	2	2
V _{ss} (L/kg)	3.8	4.8	---	---
Cl (ml/min/kg)	37	42	---	---
T _{max} (minutes)	---	---	75	75

These data indicated similar pharmacokinetics for the S- and R-enantiomers of SB-265805 following either *iv* infusion or oral administration of racemic RS-SB-265805. The oral bioavailability for both enantiomers is approximately 11 percent based upon AUC comparisons between the oral dosing and *iv* infusion.

Pharmacokinetic data were also generated for SB-265805 and N-acetyl SB-265805 (SB-41400) in male and female CD rats following oral doses of 210 and 750 mg SB-265805 per kg body weight. The N-acetyl metabolite of SB-265805 was shown to represent 5 to 10 % of circulating plasma radioactivity in human pharmacokinetic studies with [¹⁴C]SB-265805. The sponsor elected to evaluate the circulating levels of N-acetyl SB-265805 in rats following dose levels used in a 28-day toxicity study (210 and 750 mg/kg). The C_{max} and AUC values for both compounds are listed in the following table.

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	210 mg/kg SB-265805		750 mg/kg SB-265805	
	SB-265805			
	Male	Female	Male	Female
Cmax (µg/ml)	1.7	5.4	4.1	9.2
AUC (µg • hr/ml)	11.5	25.3	49	84
	N-Acetyl SB-265805 (SB-41400)			
Cmax (µg/ml)	0.068	0.16	0.18	0.41
AUC (µg • hr/ml)	0.36	0.77	2.18	3.9

As analytical techniques were used in this study with an LLQ of 10 ng/ml for both SB-265805 and N-acetyl SB-265805. The AUC data for SB-265805 indicated that females had approximately 2-fold greater systemic exposures than males to orally administered SB-265805. The AUC values for N-acetyl SB-265805 ranged from 3 to 5 percent of the AUC values for SB-265805 (both sexes and dose levels). The N-acetyl SB-265805 AUC values were also higher in females compared to males.

SB Study Report Number SB-265805/RSD-100V86/3: A 14-Day Oral Study in Rats to Investigate the Pharmacokinetics of SB-265805 and the Effect on Hepatic Levels of Cytochrome P450 and Related Parameters. ^{GLP}

Male and female CD rats were dosed orally for 14 days at SB-265805 dose levels of 23, 68, and 159 mg free base/kg body weight. Limited pharmacokinetic evaluations were conducted on Days 1 and 14 of dosing. Serial blood samples were collected for 24 hours after dosing. The effect of SB-265805 on hepatic cytochrome P-450 enzymes was determined at the 24-hour sacrifice following the terminal dose. The following determinations were made; liver weights, liver/body weight ratios, mg microsomal protein per g liver, and nmole Cytochrome P-450 per mg microsomal protein. The following hepatic microsomal enzyme activities were measured with corresponding cytochrome P-450 subfamilies listed in parentheses; 7-ethoxyresorufin O-deethylase (CYP1A), testosterone 2α- and 7β-hydroxylase (CYP2C11), testosterone 6β-hydroxylase (CYP3A), testosterone 7α-hydroxylase (CYP2A1), testosterone 16α-hydroxylase (CYP2C11), lauric acid 11-hydroxylase (CYP2E1), lauric acid 12-hydroxylase (CYP4A), and testosterone 17β-dehydrogenase.

The following table lists the AUC data for SB-265805 at Day 1 and Day 14 at the different dose levels for males and females.

	AUC (µg • hr/ml)			
	Day 1		Day 14	
Dose Level	Males	Females	Males	Females
23 mg/kg	1.1	1.7	0.8	1.3
67 mg/kg	3.8	3.9	3.4	4.2
159 mg/kg	6.6	15.4	7.2	12.8

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These data represent composite values from all animals at each dose level. There was no apparent effect on the AUC values following multiple doses at any of the SB-265805 dose levels. Female rats at the highest dose level (159 mg/kg) exhibited AUC values that were approximately 2-fold greater than AUC values from male rats at the same dose level.

Repeat dosing with SB-265805 did not affect liver weights, liver/body weight ratios, mg microsomal protein per gram liver, or nmoles of cytochrome P-450 per mg microsomal protein. These values were generated from four male and four female rats at each dose level including the zero-level vehicle control. These data gave no indication of hepatic microsomal enzyme induction as a result of repeat dosing with SB-265805. The cytochrome P-450 enzyme activities measured in this study were not altered (either induced or reduced) as a result of dosing with SB-265805. The only enzyme activity that was altered (statistically significant) was a reduction in 17 β -dehydrogenase activity in females by approximately 50 percent at the high dose level (159 mg/kg). This was indicative of reduced androstenedione production. The sponsor concluded that SB-265805 was not an inducer of hepatic cytochrome P-450 activity as a result of repeat dosing.

SB Study Report Number SB-265805/RSD-100XCM/1: [14C]SB-265805-S: Quantitative Whole-Body Autoradiography Following Single Oral Administration (210 mg/kg) to the Male Pigmented Rat. ^{GLP}

SB Study Report Number SB-265805/RSD-100XCP/2: Placental Transfer of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805-S to the Pregnant Rat at a Nominal Dose Level of 270 mg/kg (217 mg free base/kg). ^{GLP}

SB Study Report Number SB-265805/RSD-100XCR/1: Milk Secretion of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805 to the Lactating Rat at a Nominal Dose Level of 30 mg/kg (24 mg free base/kg). ^{GLP}

The sponsor submitted a series of radioactivity distribution studies in rats following single doses of [¹⁴C]SB-265805. A probe or pilot study was conducted in male pigmented rats (Lister-Hooded rats) by whole-body autoradiography at different time intervals after a 168 mg free base/kg body weight dose that contained [¹⁴C]SB-265805. Only one animal was used at each of the following timepoints; 2 and 24 hours, 3, 10, and 21 days. At the two-hour timepoint most of the radioactivity was in the gut content. The systemically absorbed radioactivity had distributed to most organs and tissues with the highest levels in liver, kidney, bone marrow, spleen; all of which were several fold higher than blood concentration. None of these tissues including blood contained detectable radioactivity by 24 hours after dosing (as determined by whole-body autoradiography). The uveal tract of the eye (a melanin-containing tissue in this rat strain) contained radioactivity at all timepoints with a peak intensity occurring 3 days after dosing. This observation is consistent with other fluoroquinolones which persist in tissues containing melanin pigment.

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Studies were conducted with pregnant and lactating female rats (Sprague Dawley) to determine the amount of [^{14}C]SB-265805 that was placentally transferred to the fetus and the concentration secreted in the milk of lactating female rats. Pregnant Sprague Dawley rats received a single oral dose of [^{14}C]SB-265805 at a dose level of 217 mg/kg on Day 18 of gestation. Three rats comprised each of the following post-dosing timepoints; 0.5, 6, 12, 24, and 76 hours post-dosing. Radioactivity concentrations were determined in the following; amnionic fluid, placenta, fetal tissue, and maternal liver, kidney, plasma, mammary tissue, ovaries, and uterus. The following table lists the average μg equivalents per gram tissue values for a selected group of these tissues.

	μg Equivalents per Gram Tissue			
	0.5 Hour	6 Hours	12 Hours	24 Hours
Plasma	4.8 ± 2	2.1 ± 0.6	0.3 ± 0.1	---
Liver	45 ± 20	20 ± 7	2.3 ± 0.9	2.7 ± 2.6
Kidney	40 ± 17	25 ± 5	2.5 ± 0.8	2.4 ± 2.4
Amnionic Fluid	0.6 ± 0.4	1.2 ± 0.2	$0.3 \pm .04$	---
Placenta	6.1 ± 3.2	8.4 ± 1.3	2.5 ± 0.8	1.9 ± 1.4
Fetal Tissue	2.4 ± 1.6	2.5 ± 0.4	0.5 ± 0.2	---

These data indicated that radioactivity derived from [^{14}C]SB-265805 was placentally transferred to the developing fetus. Fetal and placental concentrations (expressed as μg equivalents of SB-265805 per gram tissue) remained essentially constant between 0.5 and 6 hours after oral dosing of [^{14}C]SB-265805. The fetal tissue/maternal plasma ratio increased between 0.5 and 12 hours after dosing with ratios of 0.5, 1.2, and 1.7 for the 0.5, 6, and 12 hour timepoints, respectively. By 24 hours after dosing there was no detectable radioactivity in maternal plasma and fetal tissue, however quantifiable levels of radioactivity were detected in the placenta, maternal liver, and maternal kidney.

Secretion of ^{14}C radioactivity derived from [^{14}C]SB-265805 was evaluated in lactating female rats on Day 13 post-partum. A single oral dose of 24 mg [^{14}C]SB-265805 per kg body weight was administered to 15 lactating female rats. Milk and blood samples were taken at five post-dosing timepoints (0.5, 6, 12, 24, and 72 hours) with three females at each timepoint. Concentrations of [^{14}C]SB-265805 equivalents per ml in milk were 7.5, 1.1, and 0.4 μg equivalents per ml at the 0.5, 6, and 12 hour timepoints, respectively. The corresponding plasma concentrations were 0.56, 0.08, and <0.04 μg equivalents per ml. Quantifiable radioactivity was not present in plasma and milk samples at the 24-hour timepoint. These data indicated that concentrations of [^{14}C]SB-265805-derived radioactivity were substantially greater in milk than plasma during the 12-hour post-dosing period.

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SB Study Report Number SB-265805/RSD-1011R4/1: Biliary and Plasma Concentrations of Drug-Related Material Following Single and 5-Day Repeat Intravenous Administration of [¹⁴C]SB-265805-S to Male Bile Duct-Cannulated Beagle Dogs at a Target Dose of 30 mg free base/kg/day. ^{GLP}

SB Study Report Number SB-265805/RSD-1011XJ/1: An Investigation of the Plasma Concentrations of SB-265805 and the Biliary Metabolite Profiles Following Single and 5-Day Repeat Intravenous Administration of [¹⁴C]SB-265805-S to Male Bile-Duct Cannulated Dogs at a Target Dose of 30 mg free base/kg. ^{GLP}

Bile-duct cannulas were inserted into three adult male beagle dogs for the purpose of assessing the biliary secretion of radioactive compounds derived from [¹⁴C]SB-265805. Each dog received an intravenous dose of 30 mg [¹⁴C]SB-265805/kg body weight for five consecutive days. Serial blood samples were drawn for a six-hour period following the iv infusion after the first and fifth doses at 5, 10, 15, 30, and 45 minutes, 1, 1.5, 2, 3, 4, 5, and 6 hours after the inception of the iv dose. Bile was collected at the same time as blood was drawn according to the following schedule; 0 to 5 minutes, 5 to 10 minutes, 10 to 15 minutes, 15 to 20 minutes, 20 to 25 minutes, 25 to 30 minutes, 30 to 45 minutes, 45 to 60 minutes, 60 to 90 minutes, 90 to 120 minutes (2 hours), 2 to 3 hours, 3 to 4 hours, 4 to 5 hours, and 5 to 6 hours. This sequence quantitatively collected bile secretion for six hours after the initiation of the iv infusion.

All plasma and bile samples were assayed for total ¹⁴C radioactivity levels. The data were quantitated and expressed as µg equivalents of [¹⁴C]SB-265805. Plasma samples were subsequently analyzed by _____ techniques to quantitate for SB-265805 and to develop pharmacokinetic profiles. Bile samples were initially analyzed by _____ detection to generate metabolite profiles and then by _____ to confirm metabolite structures by comparison to chemically synthesized metabolites. In addition to the metabolism and pharmacokinetic portion of the two studies, serum chemistry was conducted on blood samples drawn prior to cannulation surgery, before dosing, and during the dosing routine. The following were assayed; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), sorbitol dehydrogenase (SDH), and total bilirubin.

Plasma AUC_{0 → 6 hr} values averaged 38 µg equivalents • hr/ml on Day 1 of dosing and 44 µg equivalents • hr/ml after dosing on Day 5. The differences were not statistically significant. The plasma SB-265805 AUC_{0 → 6 hr} values were 23 and 21 µg • hr/ml on Day 1 and Day 4, respectively. Therefore, SB-265805 represented approximately 61 percent of the plasma AUC expressed as total radioactivity on Day 1 and 48 percent on Day 5. The metabolite of SB-265805 measured in plasma was the acyl glucuronide of SB-265805 which represented 3 to 5 percent of the total plasma radioactivity in the early post-dosing timepoints (0 to 2 hr plasma pool) and 9 to 11 percent in the latter timepoints (3 to 6 hour pool). The remaining plasma radioactivity was not characterized by the sponsor in these two reports.

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Several pooled biliary timepoints were assessed by _____, detection and by _____ detection/_____. The major biliary component was [^{14}C]SB-265805 which averaged 36 to 46 percent of the total radioactivity in selected sample pools (25 to 30 minutes, 45 to 60 minutes, 90 to 120 minutes, and 300 to 360 minutes) from bile collections on Day 1 and 44 to 63 percent from bile collections at the same time intervals on Day 5. The most abundant biliary metabolite of SB-265805 was its acyl glucuronide which represented 16 to 24 and 6 to 14 percent of the biliary radioactivity on Day 1 and Day 5, respectively. The only other identified SB-265805 biliary metabolite was O-desmethyl SB-265805 which represented from 1 to 5 percent of the total biliary radioactivity. Four additional radioactive metabolite peaks exhibiting baseline resolution were noted in the _____ chromatographs presented by the sponsor. Each of these peaks appeared to match the same relative abundance as O-desmethyl SB-265805. Other peaks which did not achieve baseline resolution were also observed.

Serum chemistry indicated hepatic effects to 2 of the 3 dogs in this study. The AST and ALT levels were increased from 7- to 127-fold by Day 5 of dosing (compared to the baseline values established after surgery and prior to the initial dose). The ALP and GGT levels were increased 8- to 15-fold in the same two dogs while SDH levels were increased 19- to 48-fold. Total bilirubin was elevated 4-fold in 1 of 3 dogs.

SB Study Report Number SB-265805/RSD-100SKR/2: A Preliminary Study to Investigate the Absorption, Excretion, and Biliary Secretion of Drug-Related Material in the Dog Following a Single Oral Administration of [^{14}C]SB-265805 at a Target Dose of 24 mg free base/kg.

SB Study Report Number SB-265805/RSD-100XCK/1: Elimination of Drug-Related Material Following Single Doses of [^{14}C]SB-265805-S to Male and Female Dogs. ^{GLP}

The preliminary, or probe, absorption and excretion study consisted of non-cannulated male and female beagle dogs and bile-duct cannulated female beagle dogs, all receiving a single oral dose of 24 mg [^{14}C]SB-265805 per kg body weight. Urine and feces were collected for 120 hours after dosing from non-cannulated animals and for 96 hours after dosing from the bile-duct cannulated female dogs. Bile was quantitatively collected at the following time intervals after dosing; 0 to 6 hours, 6 to 12 hours, 12 to 24 hours, and at 24-hour intervals thereafter. Excretion data from the non-cannulated animals indicated 14 percent of the administered radioactivity was eliminated in urine and approximately 78 percent in feces. Bile-duct cannulated dogs excreted 18 percent of the administered radioactivity in urine and 46 percent in feces. Secretion into the bile accounted for 25 percent of the administered radioactive dose. Addition of the urinary and biliary radioactivity indicated that approximately 43 percent of the orally administered dose was absorbed into systemic circulation.

_____, detection and _____, detection/_____ analysis were conducted on the urine and bile samples and fecal extracts. Urine samples from cannulated and non-cannulated animals contained unchanged SB-265805 which

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represented 69% of the total urinary radioactivity. Other urinary metabolites included the acyl glucuronide of SB-265805 (7 to 10%), the E-isomer of 265805 (10%), the acyl glucuronide of N-acetylated SB-265805 (2%), and N-acetylated SB-265805 (<1%). The fecal extraction methods resulted in only 59% of the total radioactivity in feces being ———-analyzed. Unchanged SB-265805 represented 40% of the total fecal radioactivity with the E-isomer of SB-265805 accounting for approximately 4%. Hydroxymethyl SB-265805 and N-acetyl 265805 each represented less than 1% of the extracted radioactivity.

The biliary metabolite pattern was more complicated with at least seven minor radioactive metabolite peaks that were not identified. The acyl glucuronide of SB-265805 was the most abundant metabolite and represented 43% of the total biliary radioactivity. Unchanged SB-265805 and its E-isomer together accounted for approximately 20% of the biliary radioactivity. Additional biliary metabolites included the acyl glucoside of SB-265805 (5%), acyl glucuronide of hydroxymethyl SB-265805 (4%), N-acetyl SB-265805 (1%), and hydroxymethyl SB-265805 (1%). The radioactivity metabolite profile of plasma samples indicated that unchanged SB-265805 represented approximately 60% of the total plasma radioactivity while its E-isomer accounted for 3 to 6%. The other identified plasma metabolite peaks were the acyl glucuronide of SB-265805 (3 to 5%) and hydroxymethyl SB-265805 (1.5%). Approximately 25% of the plasma radioactivity was not extracted by the plasma preparation procedures outlined in the report and consequently not ———-analyzed.

The routes of excretion for radioactive compounds derived from [^{14}C]SB-265805 were evaluated following a single oral 24 mg/kg dose to male and female dogs, a single 10 mg/kg *iv* dose to bile-duct cannulated male dogs, and a single oral 24 mg/kg dose to bile-duct cannulated male dogs. The accuracy of the study was compromised because all of the male and female dogs receiving the 24 mg/kg oral dose vomited within 6 hours post-dosing. The percent of dose that was vomited ranged from ——— percent for males and 4 to 16 percent for females. The average percent of dose excreted in feces was 60 percent for males and 66 percent for females while urinary excretion averaged approximately 18 percent for both sexes. Over 94 percent of the total fecal radioactivity was eliminated by 48 hours after dosing and approximately 90 percent of the total urinary radioactivity was excreted by 24 hours post-dosing.

Male dogs (bile-duct cannulated) dosed *iv* excreted 27, 39, and 30 percent of the radioactive dose in urine, feces, and bile, respectively. The same animals were subsequently dosed orally at 24 mg/kg dose level (following a wash out period of one month). The average excretion percentages were 9.6, 76, and 8.4 for the urine, feces, and bile, respectively. This study indicated substantial secretion of radiolabelled metabolites from the intestinal epithelium into the lumen of the G.I. tract following the *iv* dose. The percent intestinal absorption indicated by the oral dose was 18 percent (urinary plus biliary percent values), however this probably underestimates absorption of [^{14}C]SB-265805 into systemic circulation due to secretion of ^{14}C -labelled metabolites from the intestinal epithelium into the lumen of the G.I. tract (and subsequent fecal elimination).

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SB Study Report Number SB-265805/RSD-100XFZ/1: Biotransformation of [^{14}C]SB-265805 in Rat and Dog. ^{GLP}

The sponsor retrieved urine, bile, plasma, and fecal samples from rats and dogs dosed with [^{14}C]SB-265805 in previously cited studies for _____ analysis. The purpose was to develop complete metabolite profiles based upon _____ detection for each sample and then to analyze each metabolite by _____. The following authentic standards of probable metabolites of SB-265805 were used to confirm metabolite structures.

N-acetylated Analog of SB-265805 (SB-41400, N-acetyl SB-265805)	(+/-)-7-[4-(acetylaminoethyl)-3-(Z)-methoxyimino] pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid
Demethylated Analog of SB-265805 (SB-413006, O-desmethyl SB-265805)	(+/-)-7-[3-aminomethyl)-(4-(Z)-methoxyimino) pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid
Deaminated Analog of SB-265805 (SB-414007, Deaminated SB-265805)	(+/-)-7-[3-(hydroxymethyl)-(4-(Z)-methoxyimino) pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid

The acyl glucuronide of SB-265805 was confirmed by _____ analysis and _____ of _____ purified metabolite. The structures of several other metabolites present in the various biological samples from rats and dogs were proposed based upon mass number, parent and daughter ions from _____ analysis, _____ comparisons to other authentic standards, and _____ retention times.

The biological samples were from non-cannulated male and female rats dosed orally at 168 mg [^{14}C]SB-265805 per kg body weight and bile-duct cannulated male rats at the same dose level; bile-duct cannulated male rats dosed by *iv* administration at a dose level of 10 mg [^{14}C]SB-265805 per kg body weight; non-cannulated male and female dogs dosed orally at 24 mg [^{14}C]SB-265805 per kg body weight and bile-duct cannulated male dogs dosed at the same dose level; and bile-duct cannulated male dogs dosed by *iv* administration at a dose level of 10 mg [^{14}C]SB-265805 per kg body weight.

The following table lists the confirmed and proposed metabolites of SB-265805 and the relative percent of each metabolite in the _____ analyzed sample. The percentage data encompass the values from males and females dosed orally or by *iv* administration to non-cannulated and bile-duct cannulated animals. The metabolites listed in bold type were structurally confirmed by _____ comparisons to authentic standard or by _____ analysis.

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Gemifloxacin Metabolite Chart for Rats and Dogs

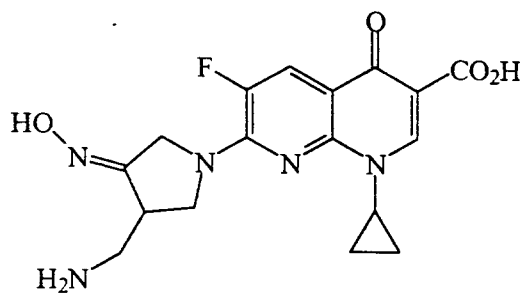
METABOLITES	Relative Percent Distribution of Radioactive Metabolites							
	Urine		Fecal Extract		Bile		Plasma	
	Dog	Rat	Dog	Rat	Dog	Rat	Dog	Rat
SB-265805	73 to 80	60 to 70	40 to 70	50 to 70	34	15 to 16	50 to 60	50 to 70
E-isomer SB-265805	6 to 11	7 to 9	3 to 6	3 to 4	2 to 4	1	5 to 9	8 to 13
N-Acetyl SB-265805	---	2 to 4	---	1 to 7	---	6 to 7	---	2 to 3
Acyl Glucuronide of SB-265805	3 to 5	1 to 2	---	---	20 to 22	32 to 38	2 to 5	2 to 6
Glutamic Acid Conj. of SB-265805	---	1	---	---	---	3 to 4	2 to 4	---
Deaminated SB-265805	---	---	1	2	3 to 4	1 to 3	1 to 2	1 to 5
Acyl Gluc. Of N-Acetyl SB-265805	---	---	---	1	---	1 to 2	---	---
Acyl Gluc. Of Deaminated SB-265805	---	---	---	---	2	3 to 7	---	---
Glutamic Acid Conj. of N-Acetyl SB-265805	---	1	---	---	---	---	---	---
Acyl Gluc. Of E-isomer of SB-265805	---	---	---	---	---	3 to 4	---	---
O-Desmethyl SB-265805	1 to 3	---	---	---	6 to 8	---	---	---
Acyl Glucoside of SB-265805	---	---	---	---	5	---	---	---
Carboxylic acid of SB-265805	---	---	---	---	5 to 7	---	---	---

These data indicated that for rats and dogs unchanged SB-265805 was the major excretion product in urine samples and fecal extracts. The E-isomer of SB-265805 was also eliminated in urine and feces from both species. Identification of the E-isomer was based upon ——— identical to SB-265805 but with distinctly different ——— retention times. The acyl glucuronide of SB-265805 was prevalent in bile from rats and dogs and was also observed in plasma and urine but was not detected in fecal extracts. Its prevalence in bile but not in fecal extracts may be indicative of hydrolysis of the glucuronide conjugate by bacterial glucuronidase enzymes in the gut microflora.

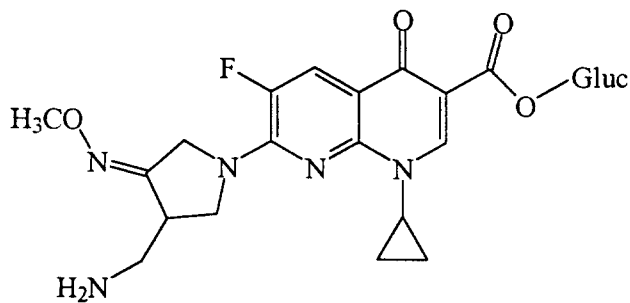
Deaminated SB-265805 was observed in plasma, bile, and fecal extracts from rats and dogs, whereas O-desmethyl SB-265805 was only observed in bile and urine from dogs. N-acetyl SB-265805 was observed in all biological samples from rats but was not detected in dog samples probably because of the low levels of N-acetylating enzymes in dogs. Additional metabolite peaks were observed in small amounts, primarily in bile from both species. These metabolites were only tentatively identified due to an absence of appropriate chemical standards and the low relative abundance that each represented.

Structural representations of identified metabolites of SB-265805 are presented in the subsequent figures.

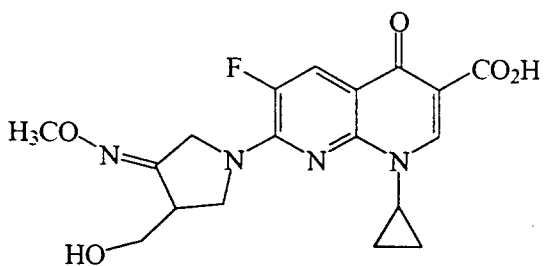
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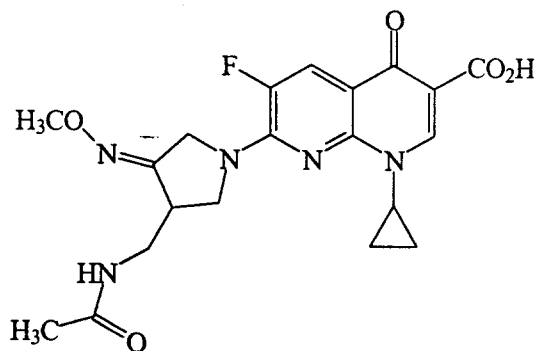
O-Desmethyl SB-265805



Acyl Glucuronide of SB-265805



Deaminated SB-265805



N-Acetyl SB-265805

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SB Study Report Number SB-265805/RSD-100T6Z/1: An Investigative Study to Determine the Pharmacokinetics of the R- and S-Enantiomers of SB-265805 Following both Intravenous and Oral Administration of SB-265805-S at a Nominal Dose Level of 10 mg/kg (as the free base) to the Male Beagle Dog. ^{GLP}

SB Study Report Number SB-265805/RSD-100WK7/1: A Study in the Beagle Dog to Determine the Intravenous Pharmacokinetics of SB-265805 Following a 30 Minute Intravenous Infusion of SB-265805 at Nominal Dose Levels of 10 and 30 mg/kg (as the free base). ^{GLP}

SB Study Report Number SB-265805/RSD-1013MS/1: A Study in the Beagle Dog to Determine the Concentrations in Cardiac Tissue Following a 30 Minute Intravenous Infusion of SB-265805 at a Nominal Dose Level of 30 mg/kg (as the free base). ^{GLP}

Pharmacokinetics for the R- and S-enantiomers of SB-265805 were evaluated in male beagle dogs following oral and iv 10 mg/kg doses of SB-265805 (racemic mixture). The R- and S-enantiomers were analyzed by _____ procedures using a chiral _____ column as described previously (SB Study Report Number SB-265805/RSD-100MZG/1). The same animals were used for oral and iv dosing following an appropriate wash out period. The following table lists the R- and S-enantiomer AUC values from each animal.

AUC Values (ng • hr/ml)					
Oral Dose (10 mg/kg)					
Enantiomer	Dog #1	Dog #2	Dog #3	Dog #4	Dog #5
S	5140	1840	2930	7690	---
R	5310	1940	3490	7670	---
Intravenous Dose (10 mg/kg)					
S	---	7000	6000	8050	5330
R	---	7000	6170	7670	4960

Dog #1 did not receive the iv dose and Dog #5 did not receive the oral dose. The AUC values for the S- and R-enantiomers were equivalent for each dog regardless of the route of administration of SB-265805. These data revealed large (approximately 4-fold) animal to animal variations for AUC values following oral dosing. Large variations were also observed for C_{max} values after oral dosing with an average C_{max} of 700 ng/ml (S- and R-enantiomers) with a range of values from 300 to 1000 ng/ml. The plasma half-life values were independent of the isomeric form and route of administration with average values ranging from 5 to 6 hours. The remaining pharmacokinetic values (volume of distribution and plasma clearance) for each isomer were similar for each individual dog.

The pharmacokinetic patterns of SB-265805 were also evaluated in male beagle dogs after iv administration of two different dose levels of SB-265805 (10 and 30 mg/kg). SB-265805 plasma levels were quantitated using _____ procedures. The LLQ for this procedure was approximately _____ ng/ml; this procedure did not separate the R- and S-enantiomers. The average AUC values were 12.8 and 38.9 µg • hr/ml for the 10 and 30 mg/kg doses, respectively, and were proportional to the dose levels. The plasma half-

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lives for SB-265805 were approximately 5 to 6 hours and were independent of dose level. The volume of distribution at steady state (V_{ss}) was approximately 5 L/kg and the total plasma clearance averaged 0.8 (L/hr)/kg. Both measures were independent of dose level.

In a companion study with male beagle dogs, plasma SB-265805 concentrations were compared to tissue concentrations in the heart at the termination of a 30 minute *iv* infusion of SB-265805 at a 30 mg/kg dose level. The average plasma concentration was 6.2 µg/ml compared to an average concentration in heart tissue of 51 µg/g. The individual heart/plasma SB-265805 concentration ratios for the three animals used in this study were 9.0, 5.5, and 10.0. These data indicated a modest accumulation of SB-265805 in heart tissue during the 30 minute *iv* infusion of SB-265805 at the 30 mg/kg dose level.

SB Study Report Number SB-265805/RSD-100XG0/2: The Effect of SB-265805-S on Hepatic Levels of Cytochrome P450 and Related Parameters in Beagle Dogs after Oral Administration at 0, 5, 30, and 120 mg/kg/day for 13 Weeks. ^{GLP}

Male and female beagle dogs (four of each sex at each dose level) received single daily oral doses of SB-265805 for 13 weeks at dose levels of 0, 4, 24, and 96 mg free base of SB-265805 per kg body weight. The highest dose was reduced on Day 41 to 48 mg/kg due to unspecified toxicity observed in all of the animals at the 96 mg/kg dose level. All animals were sacrificed 24 hours after the terminal dose; liver samples were taken and microsomal pellets were subsequently isolated. Microsomal protein levels were quantitated and expressed as mg microsomal protein per gram liver. Cytochrome P-450 levels were determined and expressed as nmole Cytochrome P-450 per mg microsomal protein. Several enzymatic activities mediated by different subfamilies of Cytochrome P-450 were determined and are listed below.

<u>Enzymatic Activity</u>	<u>Cytochrome P-450 Subfamily</u>
7-Ethoxyresorufin O-deethylase	CYP1A (1/2)
Testosterone 6β-hydroxylase	CYP3A12
Testosterone 16α-hydroxylase	CYP2C/2B11
Testosterone 16β-hydroxylase	CYP2B
Testosterone 2β-hydroxylase	CYP3A
Lauric acid 11-hydroxylase	CYP2E
Lauric acid 12-hydroxylase	CYP4A

Testosterone 17β-dehydrogenase activity was also assessed by quantitating androstenedione formation.

None of the SB-265805 dose levels affected the values for mg microsomal protein per gram liver and nmole Cytochrome P-450 per mg microsomal protein when compared to values from the zero-level vehicle controls. None of the Cytochrome P-450 mediated enzyme activities were induced by any SB-265805 dose level. The activity levels for 7-ethoxyresorufin O-deethylase, testosterone 6β-hydroxylase, and testosterone 2β-hydroxylase were marginally lower in hepatic microsomal preparations from animals at the mid-dose level (24 mg/kg) compared to the activity from the zero-level vehicle

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controls, however these differences were not statistically significant. Under conditions of this study repeat oral dosing with SB-265805 at three different dose levels did not result in the elevation of hepatic microsomal protein, nmoles of Cytochrome P-450, and enzymatic activity associated with different subfamilies of Cytochrome P-450.

SB Study Report Number SB-265805/RSD-XFX/1: A Preliminary in vitro Investigation into the Biotransformation of SB-265805.

SB Study Report Number SB-265805/RSD-100W5S/1: Characterisation of the Metabolism of [¹⁴C]SB-265805 in Whole Cell Liver Tissue Models.

SB Study Report Number SB-265805/RSD-100XCV/1: The Metabolism of [¹⁴C]SB-265805 in Whole Cell Liver Systems from Rabbit and Mouse. ^{GLP}

SB Study Report Number SB-265805/RSD-1013M3/1: An in vitro Investigation into the N-Acetylation of SB-265805. ^{GLP}

SB Study Report Number SB-265805/RSD-10158N/1: A Preliminary Investigation into the Cytochrome P450 Inhibitory Potential of SB-265805.

SB Study Report Number SB-265805/RSD-100ZHK/2: Evaluation of Racemic SB-265805 and its Individual Enantiomers as Inhibitors of Human P450 Enzymes in vitro. ^{GLP}

A series of *in vitro* metabolism studies were conducted by the sponsor to determine the extent to which SB-265805 was metabolized and to generate metabolite profiles. Initial studies with SB-265805 (racemic mixture), and the purified R- and S-enantiomers indicated that the racemic mixture and purified enantiomers yielded the same metabolic profiles following incubation with freshly isolated hepatocytes from rat, dog, and human sources. The N-acetyl derivative of SB-265805 was generated by hepatocytes from rats and humans but not from dogs, whereas the acyl glucuronide of SB-265805 was produced by all hepatocyte sources.

A more extensive *in vitro* study was conducted using [¹⁴C]SB-265805 in freshly isolated rat, dog, and human hepatocytes. Human liver slices were also evaluated. After a 24-hour incubation period the supernatants were analyzed by _____ detection procedures and subsequently by _____. Unchanged SB-265805 represented from 69 to 83 percent of the _____ metabolite profiles from the different hepatocyte sources. The E-isomer of SB-265805 represented from 10 to 12 percent, therefore 79 to 95 percent of the [¹⁴C]SB-265805 substrate was not metabolized during the 24-hour incubations. N-acetyl SB-265805 represented 5 and 8 percent of the rat and human metabolites, respectively. N-acetyl SB-265805 was not produced by dog hepatocytes.

Comparative metabolite profiles from [¹⁴C]SB-265805 were also generated from freshly isolated hepatocytes from CD and hairless mice and New Zealand white rabbits. After 24 hour incubations unchanged SB-265805 represented 70 to 75 percent of the radioactive

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metabolites from the mouse hepatocytes with the E-isomer consisting of 10 to 12 percent of the total analyzed radioactivity. Rabbit hepatocytes metabolized [¹⁴C]SB-265805 to a greater extent than mouse hepatocytes with unchanged SB-265805 representing 57 percent of the analyzed radioactivity. The percent represented by the E-isomer of SB-265805 ranged from 10 to 12 percent for the mouse incubations and 8 percent for the rabbit incubations. N-acetyl SB-265805 was detected in the mouse incubations (approximately 2 percent of the analyzed radioactivity) but was not observed in analysis of the rabbit incubation supernatant. Conversely, O-desmethyl SB-265805 was detected in the rabbit incubation supernatant (approximately 10 percent of the total) but was not formed by mouse hepatocytes. SB-265805 was deaminated by hepatocytes from mice and rabbits yielding a carboxylic acid metabolite from both mouse and rabbit hepatocytes (approximately 3 and 6 percent of the analyzed radioactivity, respectively). In addition, an alcohol derivative of deaminated SB-265805 was produced by rabbit hepatocytes (approximately 6 percent of the analyzed radioactivity).

The formation of N-acetyl SB-265805 was evaluated in human liver cytosolic preparations from 46 different human liver samples. SB-265805 was incubated for 30 minutes in the presence of human liver cytosol and Acetyl Co-A. Concurrent incubations were conducted with two model or probe substrates, *p*-aminobenzoic acid and sulfamethazine, which were used to quantitate human N-acetyl transferase 1 and 2 (NAT1 & 2) activities, respectively. N-acetyl SB-265805 was formed under these incubation conditions at rates ranging from 0.6 to 9 pmoles/min/mg protein. The acetylation rates for the two model substrates were three orders of magnitude greater than the acetylation rates for SB-265805. The NAT2 isoform appeared to be primarily responsible for the N-acetylation of SB-265805 based upon correlation to the acetylation of the sulfamethazine substrate in the different human liver samples.

A pilot Cytochrome P-450 inhibition study indicated that SB-265805 (racemic mixture), and the purified R- and S-enantiomers of SB-265805 in general did not inhibit different subfamilies of human derived Cytochromes P-450. The following enzyme activities were evaluated; caffeine N3-demethylase (CYP1A2), Naproxen O-demethylase (CYP2C9), Diazepam N-demethylase (CYP2C19), Dextromethorphan O-demethylase (CYP2D6), and Dextromethorphan N-demethylase (CYP3A4). There was a modest reduction of Dextromethorphan N-demethylase activity ranging from 26 to 53% in the presence of 100 μ M SB-265805.

The inhibition potential of SB-265805 (racemic mixture) and the purified R- and S-enantiomers was subsequently examined in detail with human hepatic microsomal preparations. The model or probe substrates were evaluated at their various *K_m* concentrations. The inhibition concentration for SB-265805 (racemic mixture and purified enantiomers) was experimentally determined to be 100 μ M. The incubation conditions such as microsomal protein concentration, linearity of product formation, and incubation time were experimentally determined. The following table lists the probe substrates used to reflect the activities of the different subfamilies of human Cytochromes P-450.

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<u>Probe Enzymatic Activity</u>	<u>Cytochrome P-450 Subfamily</u>
7-Ethoxyresorufin O-dealkylation	CYP1A2
Coumarin 7-hydroxylation	CYP2A6
Diclofenac 4'-hydroxylation	CYP2C9
S-Mephenytoin 4'-hydroxylation	CYP2C19
Dextromethorphan O-demethylation	CYP2D6
Chlorzoxazone 6-hydroxylation	CYP2E1
Testosterone 6 β -hydroxylation	CYP3A4/5
Lauric acid 12-hydroxylation	CYP4A9/11

The racemic mixture of SB-265805 and each of the purified enantiomers exhibited no significant inhibition of any of the probe substrate activities. SB-265805, therefore, did not appreciably inhibit any human Cytochrome P-450 activity.

SB Study Report Number SB-265805/RSD-100XCN/2: The in vitro Blood/Plasma Partitioning of [¹⁴C]SB-265805 in rat, dog, and man. ^{GLP}

SB Study Report Number SB-265805/RSD-100T54/1: The in vitro Protein Binding of the R and S Enantiomers of SB-265805 in Rat, Mouse, Dog, and Human Plasma. ^{GLP}

SB Study Report Number SB-265805/RSD-1010V9/1: Investigation of SB-265805 (Fluoroquinolone) Permeability in vitro Across Various Intestinal Models.

The sponsor determined that [¹⁴C]SB-265805 equilibrated between whole blood cells and plasma in the following blood/plasma ratios; 1.8 for rats and dogs, and 1.2 for human blood. Plasma protein binding studies with SB-265805 and plasma samples from mice, rats, dogs and humans indicated plasma protein binding of 57 % for mice, 59 % for dogs, 68 % for humans, and 75 % for rats. The R- and S-enantiomers exhibited similar plasma protein binding percentages based upon _____ analysis with _____ columns.

The diffusion of SB-265805 across intestinal epithelium was evaluated *in vitro* with epithelium sections from rats (ileum and distal colon) and rabbits (ileum and distal colon). _____ technique was used to determine the mucosa to serosa and serosa to mucosa permeability rate. The serosa to mucosa rates ranged from 0.09 to 0.10 cm/hr for both rat and rabbit preparations. These rates were substantially greater than the companion mucosa to serosa rates (94-fold for rabbit ileum, 12-fold for rat distal colon, 9.4-fold rat ileum, and 7.7-fold for rabbit distal colon).

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EVALUATION AND CONCLUSIONS

The sponsor submitted several reports that validated analytical procedures for quantitating SB-265805 levels in plasma samples from several laboratory animal species. Plasma sample preparation procedures quantitatively extracted SB-265805 for analysis. analysis was the most sensitive and selective for SB-265805 with an LLQ of ng SB-265805/ml plasma and a range of linear detection from to ng SB-265805/ml plasma. This level of sensitivity enabled the sponsor to quantitate plasma SB-265805 levels for longer periods of post-dosing time in pharmacokinetic studies. Additionally, the sponsor provided similar analytical validation for the N-acetyl conjugate of SB-265805.

SB-265805 exists as a racemic mixture of the R- and S-enantiomers. The sponsor developed and validated an procedure to separate and quantitate each isomer. Baseline separation and resolution were achieved by using a column. The LLQ for each enantiomer was ng/ml plasma. This analytical procedure enabled the sponsor to develop pharmacokinetic data for each enantiomer following dosing with the SB-265805 racemic mixture and also to determine the extent to which interconversion occurred between the two enantiomers.

Pharmacokinetic analyses for SB-265805 were provided for rats, mice, and dogs under different dosing routines. The sponsor determined SB-265805 C_{max} and AUC values in mice following a single 50 mg/kg *ip* dose in order to establish the systemic exposure experienced by mice at an SB-265805 dose level used in a previously conducted assay. The C_{max} and AUC values for male and female mice were 4.2 µg/ml and 11.5 µg • hr/ml, respectively. These data were generated from a sufficient number of post-dosing plasma timepoints to provide an accurate AUC determination.

SB-265805 pharmacokinetic data were generated in rats following different dosing routines. Plasma AUC values for SB-265805 were determined in male and female rats following single oral doses of 210 and 750 mg/kg (dose levels used in a 28-day toxicity study). The AUC data indicated 2-fold greater systemic exposure to SB-265805 for female rats at each dose level compared to male rats (11.5 vs 25.3 µg • hr/ml at the 210 mg/kg dose and 49 vs 84 µg • hr/ml at the 750 mg/kg dose). The increase in AUC values was proportional to the dose level increase. Plasma AUC values were also determined for N-acetyl SB-265805 and were from 3 to 5 percent as large as the respective AUC values for SB-265805. In a repeat dose study (23, 67, and 159 mg/kg for 14 days) SB-265805 plasma AUC values were similar for the respective (dose level and sex) Day 1 to Day 14 comparisons. Sex differences in AUC values were observed at the highest dose examined (159 mg/kg) with Day 1 values of 6.6 and 15.4 µg • hr/ml for males and females, respectively. The increases in AUC values were proportional to the increase in dose levels.

The R- and S-enantiomers exhibited similar pharmacokinetic patterns following single oral or *iv* doses of SB-265805 (racemic mixture) to jugular-vein cannulated male rats. The sponsor demonstrated that pharmacokinetic parameters such as volume of

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